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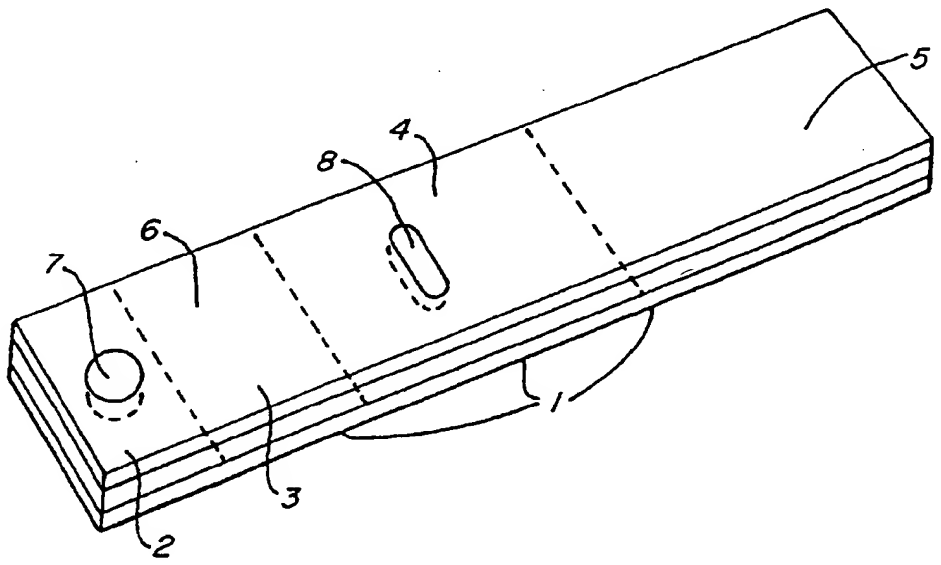
(54) Title: ANTIGEN-SPECIFIC ONE-STEP ASSAYS




(57) Abstract

A method and a device for determining, in a biological fluid sample, levels of antigen-specific immunoglobulins, said device comprising a matrix (1) defining an axial flow path, which matrix (1) has a sample receiving zone (2), a labelling zone (3) comprising a visible label bound to a first immunoglobulin-binding substance, a capture zone (4) comprising an immobilized second immunoglobulin-binding substance, and an absorbent pad (5) located sequentially downstream therein. Application of the sample to the sample receiving zone (2) results in specific binding of immunoglobulin present in the sample to labelling complex and accumulation of any antigen-specific immunoglobulin-labelling complex in the capture zone (4). The device and method are useful in determining allergen-specific IgE or other immunoglobulins specific for pathogens; and, may be used to simultaneously determine a plurality of immunoglobulins specific for different antigens or pathogens.

# ANTIGEN-SPECIFIC ONE-STEP ASSAYS

## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

The present invention relates generally to devices and methods for the detection of immunoglobulins in biological fluids. More particularly, the present invention provides devices and methods for one-step detection of those immunoglobulins.

### 2. Description of the Background Art

Immunoglobulins are proteins, produced by plasma cells, which are related to a wide variety of biological functions. For example, immunoglobulins contribute to immune clearance of foreign antigens. During infection with a pathogen, immunoglobulins are produced to antigens of the pathogen as a means of host defense. The production of immunoglobulins to the pathogen subsides following resolution of the infection. Two immunoglobulins, IgG and IgM, are the most abundant immunoglobulins produced in response to an infection. IgM is typically produced first, provides the initial defense against the pathogen, and is quickly cleared from the host. IgG is produced later during the course of the infection and provides a more long lasting defense against the pathogen. Another immunoglobulin, IgA, is secreted by the host to provide a surface defense mechanism.

Detecting the presence of an immunoglobulin in a biological fluid which reacts with a pathogen-specific antigen can aid or confirm a diagnosis of infection by the pathogen. For example, Helicobacter pylori (H. Pylori) antibody detection by antibody subclass reactivity measure against H. pylori species-specific antigens may be used as an aid in diagnosis of peptic ulcer disease, non-ulcer dyspepsia, and

Allergic reactions may also be mediated through immunoglobulins. Type I hypersensitivity reactions, such as hayfever and allergic rhinitis, are dependent upon IgE which reacts with the causative allergen. Atopic individuals have higher levels of IgE in their serum than non-atopic individuals. Also, IgE which is specific for the causative allergens is present in the patient's serum.

Identification of allergens to which a patient is sensitive is typically accomplished by skin tests. Small amounts of different suspected allergens are subcutaneously injected into the patient. Typically, patients respond with a "wheal and flare" to allergens responsible of the patient's symptoms. Occasionally however, the small amount of allergen injected into a patient is sufficient to produce anaphylaxis and even death. Thus, in addition to the discomfort of the injections more serious side effects can occur.

Myelomas are plasma cell malignancies which typically produce immunoglobulins. The diagnosis of myeloma is generally made by plasma electrophoresis of the patient's serum. The effect of treatment of the myeloma can be monitored by serial measurement of the immunoglobulin produced by the malignant plasma cells.

From the above examples, it is clear that detection of the presence or levels of immunoglobulins in biological fluids, such as serum, is medically important and desirable. Because of the relatively low concentrations of antigen-specific immunoglobulins in biological fluids, it is difficult to detect, much less accurately quantitate, important immunoglobulins in biological fluids. For example, IgE is typically present in human serum at concentrations only on the order of $10^{-6}$ to $10^{-4}$ mg/ml. Because normal IgE levels are too low, detection of elevated levels of IgE antibodies can be difficult although useful for safely diagnosing allergic conditions. Allergen specific IgE levels are generally lower than total IgE levels in human serum.

Complex means have been developed to detect and quantitate immunoglobulins in biological fluids such as serum.

immunoglobulins reactive with an antigen in a biological fluid sample. The biological fluid sample may be whole blood, plasma, serum, nasal secretions, sputum, salvia, urine, sweat, transdermal exudates, cerebrospinal fluid, or the like. Immunoglobulins of different subclasses of isotypes or reactive with different antigens may be detected simultaneously or individually in some embodiments of the present invention. Multiple different antigen-specific immunoglobulins may be detected from a single specimen.

Devices of the present invention generally include a means for labelling the immunoglobulins having a labelling complex comprising a visible label bound to a first immunoglobulin-binding substance; a matrix defining an axial flow path, said matrix having a sample receiving zone, a capture zone located downstream from the sample receiving zone, and an absorbent zone located downstream from the capture zone; and a second immunoglobulin-binding substance immobilized in the capture zone. Application of the sample to the sample receiving zone results in specific binding of immunoglobulin present in the serum to labelling complex in the labelling means and accumulation of antigen-specific immunoglobulin-labelling complex in the capture zone based on the amount of antigen-specific immunoglobulin initially present in the serum sample. The accumulation of the antigen-specific immunoglobulin-labelling complex in the capture zone provides a means to identify the presence of antigen-specific immunoglobulins in the sample.

Methods of the present invention generally comprise applying the sample to a sample receiving zone on a matrix having a flow path to a capture zone located downstream from the sample receiving zone, wherein the sample also flows through a means for labelling the immunoglobulins that contains a labelling complex comprising a visible label bound to a first immunoglobulin-binding substance, a second immunoglobulin-binding substance is immobilized in the capture zone, and at least one of the first immunoglobulin-binding substances and the second immunoglobulin-binding substances is

refer to the concentration of immunoglobulins in the serum of the patient as expressed in concentration (mg/ml, molarity, etc.) dilution or titer, or by the assay response in quantal (yes, no; present, absent; or quantitative terms (units, classes, etc.) or through measurement of color response by instrumentated means (reflectance, transmission, etc.). Typically, the biological fluid will be serum although other fluids such as saliva, cerebrospinal fluid, transdermal exudate, whole blood, sweat sputum, nasal secretions, urine or the like may be employed.

Generally, the devices and methods of the present invention employ lateral flow assay techniques as generally described in U.S. Patent Nos. 4,943,522; 4,861,711; 4,857,453; 4,855,240; 4,775,636; 4,703,017; 4,361,537; 4,235,601; 4,168,146; 4,094,647; co-pending application U.S.S.N. 07/639,967, European Patent Application Nos. 451,800; 158,746; 276,152; 306,772 and British Patent Application No. 2,204,398; each of which is incorporated herein by reference.

The devices employ a means for labelling the suspected immunoglobulins in the serum sample with a labelling complex, and a matrix with a sample receiving zone and a capture zone. Because of the construction of the present invention, application of the patient sample in the sample receiving zone results in specific binding of immunoglobulin present in the serum to labelling complex and accumulation of antigen-specific immunoglobulin-labelling complex in the capture zone based on the amount of antigen-specific immunoglobulin initially present in the serum sample.

The means for labelling the immunoglobulins is typically a labelling zone. The labelling zone is on the matrix which defines a flow path. The labelling zone is located between the sample receiving zone and the capture zone in the sample flow path. Thus, a serum sample placed in the sample receiving zone will flow through the labelling zone before contacting the capture zone.

Alternatively, the labelling means may be a sample receiving pad. During use of the assay device, the sample

are solubilized or dispersed into the patient serum sample, target immunoglobulins in the sample which react with the immunoglobulin-binding substance are contacted and bound by the labelling complexes prior to entering the capture zone. In this manner, the serum target immunoglobulin molecules are labelled. If any of the labelled serum target immunoglobulin molecules are retained in the capture zone of the device, the label provides a means for detection.

The matrix of the assay device will typically be capable of non-bibulous lateral flow. By "non-bibulous lateral flow" is meant liquid flow in which all of the dissolved or dispersed components of the liquid are carried at substantially equal rates and with relatively unimpaired flow laterally through the membrane, as opposed to preferential retention of one or more components as would occur, e.g., in materials capable of adsorbing or imbibing one or more components.

One non-bibulous matrix material is the high density polyethylene sheet material manufactured by Porex Technologies Corp. of Fairburn, Georgia, USA. The membrane has an open pore structure with a typical density, at 40% void volume, of 0.57 gm/cc and an average pore diameter of 1 to 250 micrometers, the average generally being from 3 to 100 micrometers. The optimum pore diameter for the membrane for use in the invention is about 10 to about 50 $\mu$m. The membranes are from a few mils (.001 in) to several mils in thickness, typically in the range of from 5 or 10 mils and up to 200 mils. The membrane may be backed by a generally water impervious layer, or may be totally free standing. Other non-bibulous membranes, such as polyvinyl chloride, polyvinyl acetate, copolymers of vinyl acetate and vinyl chloride, polyamide, polycarbonate, nylon, glass fiber, orlon, polyester polystyrene, and the like, or blends can also be used.

Bibulous materials, such as untreated paper, nitrocellulose, derivatized nylon, cellulose and the like may also be used following processing to provide non-bibulous flow. Blocking agents may block the forces which account for

an antigen which is specifically bound by the immunoglobulin. Typically, the second immunoglobulin-binding substance will be an anti-immunoglobulin antibody and the first immunoglobulin-binding substance will be the labelled antigen which specifically reacts with the sample immunoglobulin to be tested. In this manner, the first immunoglobulin-binding substance will bind the $F_v$ portion of the sample immunoglobulin while the second immunoglobulin-binding substance will bind the $F_c$ portion of the sample immunoglobulin. Like the first immunoglobulin-binding substance, the second immunoglobulin-binding substance specifically binds the immunoglobulins to be detected in the sample. As the target immunoglobulins in the sample contact the capture zone, they bind to the second immunoglobulin-binding substance and are retained in the capture zone. Since target immunoglobulins present in the sample have been labelled by the labelling complexes, retention of target immunoglobulins in the capture zone is detected by observation of visible label accumulation. The accumulation of visible label may be assessed either visually or by optical detection devices, such as reflectance analyzers, video image analyzers and the like. The accumulation of visible label can be assessed either to determine the presence or absence of label in the capture zone or the visible intensity of accumulated label which may by correlated with the concentration or titer (dilution) of antigen-specific target immunoglobulins in the patient sample. The correlation between the visible intensity of accumulated label and antigen-specific immunoglobulin concentration may be made by comparison of the visible intensity to a reference standard. Optical detection devices may be programmed to automatically perform this comparison by means similar to that used by the Quidel Reflective Analyzer, Catalog No. QU0801 (Quidel Corp., San Diego, CA). Visual comparison is also possible by visual evaluation of the intensity and a color key such as used in the Quidel Total IgE Test Catalog No. 0701 (a multi-step ELISA assay). Thus, antigen-specific immunoglobulin levels may be determined.

and the like; viruses such as hepatitis virus, and the like; flowers, such as marigold, sunflower, and the like; and weeds, such as pigweed, ragweed, Russian thistle, and the like. This list is only partial and those of skill will readily appreciate that immunoglobulins to other allergens could be detected by the methods and devices of the present invention.

Non-IgE antibodies to allergens may also be detected and quantified by the devices and methods of the present invention. Detection of these antibodies is particularly useful for assessing the efficacy of allergen desensitization. Because desensitization to an allergen results from production of IgG and IgM specific for the allergen, determination of allergen-specific IgG and IgM in a patient's serum can assess the effectiveness of desensitization prior to a direct patient challenge with allergen. Also, serial determinations of allergen-specific IgG or IgM provide a method for assessing the duration of effective desensitization.

When assessing either the efficacy or duration of desensitization, one immunoglobulin-binding substance will be the allergen or an epitope thereof. The other immunoglobulin-binding substance will be either anti-IgG or anti-IgM. Typically the level of IgG or IgM which results with the allergen will be determined.

Detection of non-IgE antibodies to specific antigens is also a useful for the assessment of passive immunity. Long-term immunoglobulin treatment has been used for the treatment of different diseases. Because antibodies are naturally catabolized, passive immunization requires repeated doses. By using the devices and methods of the present invention, the presence and/or levels of antigen-specific antibodies can be easily determined and the need for additional treatment assessed. For example, γ-globulin prophylaxis against hepatitis A can be monitored in travellers in endemic regions. Thus, repeat dosage schedules can be optimized.

In some embodiments of the present invention a plurality of immunoglobulins reactive with a plurality of

the different antigens provides a means to differentiate and detect and/or quantitate different antigen-specific immunoglobulins in the serum samples. In this embodiment, the antigens are most conveniently employed as second immunoglobulin-binding substances and immobilized in the different capture zones. Each capture zone will typically contain only one specific antigen. Anti-immunoglobulin antibodies (e.g., anti-IgG or Anti-IgM) are generally employed as the first immunoglobulin-binding substances. The choice of labels is not critical and may vary.

Devices for the simultaneous detection of immunoglobulins to several different antigens are particularly useful in screening populations for past exposure to microbial pathogens. For example a single device could provide rapid screening of past exposure to HIV, syphilis, and hepatitis B in community based sexually transmitted disease clinics. Office screening of pregnant women for past exposure to Toxoplasma gondii, Rubella virus, cytomegalovirus, herpes simplex virus, and Treponema pallidum to identify fetuses at risk for TORCHES syndrome or Helicobacter pylori, as related to peptic ulcer disease or stomach cancer could be accomplished by the present invention. Those of skill in the art will readily appreciate that simultaneous screening as described above may be useful in a wide variety of other clinical situations for a variety of pathogens or allergens.

Also provided are assays for determining the present absence, or amount at immunoglobulins specific for an antigen in a biological fluid sample. The sample will typically be serum although other biological fluids are acceptable. A sample is applied to a sample receiving zone on a matrix. The matrix has a flow path to a capture zone located down stream from the sample receiving zone.

Prior to contacting the capture zone, the sample flows through a means for labelling the immunoglobulins with a labelling complex comprised of a visible label bound to a first-immunoglobulin binding substance. The first

methods also provides a convenient means to assess the efficacy of therapy and resolution of disease. When possible, the target immunoglobulin level is determined prior to treatment and then serially thereafter. Decreasing immunoglobulin levels indicate that the condition is improving while rising or stable immunoglobulin levels indicate persistent infection and disease. Similar assays may be performed to detect immunoglobulins specific for the causative agent of Lyme Disease, Borrelia burgdorferi. Many other pathogens, such as Toxoplasma gondii, Cytomegalovirus, Herpes Simplex virus, Treponema pallidum, Chlamydia trachomatis, Mycoplasma pneumonia and Rubella virus may be conveniently detected and monitored by these assays.

The assays of the present invention are also useful for simultaneously determining the presence, absence or amounts of a plurality of immunoglobulins in a biological fluid sample which are specific for different antigens. A single sample is analyzed. The sample flows to a plurality of capture zones. Prior to contacting the capture zones the sample flows through one or more labelling means. Each labelling means contains at least one first immunoglobulin-binding substance. A single first immunoglobulin-binding substance may be employed. In this case, the first immunoglobulin-binding substance must be capable at binding to all target immunoglobulins to be detected. Alternatively, a plurality of first immunoglobulin-binding substances may be employed. As noted above, the first immunoglobulin-binding substance will be a labelled antigen which specifically reacts the sample immunoglobulin to be detected.

Typically a different antigen is immobilized in each capture zone. In this way, observation of each capture zone provides a means to differentiate and identify target immunoglobulins specific for different antigens in the fluid sample.

The accumulation of label in the capture zone may be visually compared to a reference standard such as a color key to determine the level of antigen-specific immunoglobulins in

labelled antigen-specific immunoglobulins can then bind to the immobilized antigen in the capture zone. If the fluid sample contains antigen-specific immunoglobulins (which have not been labelled), the labelled and unlabelled immunoglobulins will compete for antigen binding in the capture zone. If the fluid sample does not contain antigen-specific immunoglobulins, the labelled immunoglobulins will not be inhibited from binding with the antigen and the label color in the capture zone will be of maximum intensity. If unlabelled antigen-specific immunoglobulins are present in the fluid sample, some antigens will be bound by unlabelled antibodies and the color intensity of the retained label will be decreased. The color intensity of the retained label will diminish as the concentration of antigen-specific immunoglobulins in the sample increases. The level of antigen-specific immunoglobulins in a sample can then be determined by comparing the color intensity in the capture zone with predetermined reference standards as above.

In another aspect, the labelled antigen-specific immunoglobulins may be bound to antigens in the capture zone. If antigen-specific immunoglobulins are in the biological fluid sample, the labelled immunoglobulins will be displaced from the immobilized antigens and the color intensity of the capture zone will decrease as the fluid sample contacts the capture zone. The color change can be compared to reference standards to determine the level of antigen-specific immunoglobulins in the sample.

As in the non-competitive format, any of the competitive formats may detect multiple sample immunoglobulins specific for different antigens. The different antibodies may be detected by means of different colored labels. In some embodiments different capture zones will be employed for each different antigen-specific antibody to be detected. Alternatively, different antigen-specific antibodies may be detected in a single capture zone if a combination of the different colored labels can be distinguished from the individual labels, as with a reflectance analyzer.

the capture zone 4 by virtue of the allergen-specific IgE forming a binding link between the labelling complex and the immobilized allergen. The serum sample will continue to flow into the absorbent zone 5. Excess and unbound labelled IgE will be imbibed by the absorbent zone 5 providing a means for selectively retaining labelled allergen-specific IgE in the capture zone 4. The retained allergen-specific IgE can be detected by viewing the capture zone 4 through the result window 8. The intensity of the color of the visible label in the capture zone 4 may also provide a measure of the quantity of allergen-specific IgE in the patient's serum sample.

Figure 2 illustrates another embodiment of the present invention for the detection of allergen-specific IgE in a biological fluid sample. The device is comprised of a matrix 1 having a sample receiving zone 2, a capture zone 4, and an absorbent zone 5. The matrix 1 allows lateral flow of serum samples placed in the sample receiving zone 2. The flow path directs the sample through the capture zone 4 to the absorbent zone 5.

A solid top 6 covers the matrix 1 so that samples placed in the sample receiving zone 2 can flow to the absorbent zone 5. The solid top has a sample receiving well 7 located over the sample receiving zone 2 and an result window 8 located over the capture zone 4. The sample receiving well 7 is formed so as to accept a porous sample receiving pad 9. During use, the sample receiving pad 9 fits in the sample receiving well 7 so as to channel samples onto the sample receiving zone 2. The sample receiving pad 9 contains labelling complexes comprised of a visible label and an IgE binding substance, typically the allergen.

When the serum sample is applied to the sample receiving zone 2, the sample flows through the sample receiving pad 9 solubilizing the labelling complexes. The allergen in the labelling complexes binds allergen-specific IgE in the serum sample, thus labelling the IgE with the visible label. The labeled IgE then flows laterally to the capture zone 8. Anti-IgE specific antibody is immobilized to

## EXAMPLES

The following examples demonstrate the use of devices and methods of the claimed invention in the detection of allergen-specific IgE in patient serum samples.

Example 1

In this example, the rapidity and sensitivity of the present invention was demonstrated by the detection of serum IgE specific for timothy grass, and mite allergens bound to the capture zone.

Test devices for the timothy grass and mite allergens were generally prepared by the following method. For clarity, only the devices for detection of mite antigen-specific IgE are described, however, the devices for timothy grass were similarly constructed and used.

Label particles were produced for use in devices of the present invention. Blue polystyrene latex particles, 0.4$\mu$ diameter, obtained from Polymer Laboratories, Ltd., Essex Road Church, Stretton, United Kingdom, were coated with anti-IgE. One half ml of suspended particles were diluted to one ml in 50 mM Tris buffer, pH 8.0, and centrifuged. The particle pellet was suspended in 1.0 ml Tris buffer by sonication on ice for 10 minutes. This process was repeated, but the particles were suspended in 0.5 ml antibody coupling solution (50 mM Tris buffer, pH 8.0, containing 10 mg/ml monoclonal mouse ant-human IgE, 1.0 mg/ml methylated bovine serum albumin (mBSA)) and mixed by rotation overnight at room temperature. Following this incubation, the particles were again centrifuged and the pellet suspended in 0.5 ml blocking solution (50 mM Tris buffer, pH 8.0, containing 10 mg/ml mBSA). The suspension was again mixed by rotation at room temperature for four hours, centrifuged, and the particle pellet suspended in Tris buffer, pH 8.0, containing 1.0 mg/ml mBSA. This suspension was immediately centrifuged, and the particle pellet twice washed in the same fashion. The final pellet was then suspended in 0.5 ml buffer. Particle concentration as a percent solids was calculated by determining the absorbance of the solution at 450 mm.

allergen was diluted to various levels in horse serum. These dilutions were assayed by a radioallergosorbent test (RAST, Pharmacia Diagnostics) for human IgE specific to mite allergen, as well as the QUIDEL Allergen Screen assay (QAS, Quidel Corporation) in both the 90-Minute and 19-Hour formats. Both the RAST and QAS assays were performed according to the manufacturers instructions. These dilutions were then tested in lateral flow devices of the present invention to determine comparative sensitivity. The results are presented in Table 1.

The RAST assay was performed by incubating patient serum with cellulose disks, having allergen covalently attached, overnight at room temperature. The disks were then washed 3 times with buffer provided with the kit and incubated with an anti-IgE-enzyme conjugate. After another overnight incubation, the disks were washed again and developer added and incubated for 2 hours at 37°C. Finally, a stop solution was added, and the absorbance measured at 420 mm. The amount of IgE present in serum was directly proportional to the amount of color developed; standards of known IgE concentration were assayed to provide reference.

The QAS assay was performed by incubating patient serum either 30 minutes or overnight at room temperature with a dipstick to which allergen pads were affixed. Various allergen pads were prepared from cellulose by covalent attachment of allergen prior to assembly of the dipstick. The dipstick was washed under tap water for one minute and placed into an anti-IgE-enzyme conjugate solution and incubated for 30 minutes at room temperature. The dipstick was washed again and incubated for 30 minutes at room temperature in substrate solution. After blotting, the relative color intensity was determined using a reflectance densitometer (Diagnostic Solutions, Inc.).

The lateral flow test was assayed by applying 50 μL of patient serum to the sample receiving zone of the device. Sample flowed into the labelling zone pad, releasing label particles into the nitrocellulose membrane. If allergen-

Table 2
Specific IgE
Timothy Grass

| Serum | Dilution | Rast PRU | Rast Class | Lateral Flow Signal Time (min.) |
|---|---|---|---|---|
| ASB 279 | Neat | >17.5 | 4+ | 0.92 |
| ASB 948 | Neat | 15 | 3+ | 1.62 |
| ASB 948 | 1:4.35 | 3.5 | 3+ | 0.85 |
| ASB 948 | 1:21.7 | 0.7 | 2+ | 2.72 |
| ASB 948 | 1:43.5 | 0.35 | 1+ | 5.08 |
| QAR 12 | Neat | 0 | 0 | 0 |

Example 2

The ability of the present invention to differentiate between different antigens was demonstrated using timothy grass, and mite allergens. The allergens were bound either to the labelling complexes or the nitrocellulose capture zones. Assays were performed to detect either IgE specific for a single allergen or for the simultaneous detection of IgE specific for different allergens. In assays to simultaneously detect IgE specific for different allergens, the different allergens were bound to different capture zones. The allergens were obtained from Hollister-Steir.

The assay devices were constructed in a manner similar to those described in Example 1. Allergen was bound to both the labelling complexes and the capture zones. Timothy grass allergen was used alone on the same device, but in a different capture zone, with mite allergen. In some assays, the label for the timothy grass was a different color than the label for the mite allergen.

The results are presented in Table 3 with a comparison to tests of the serum in the Quidel QAS assays. The specificity of the assays of the present invention are clearly demonstrated.

Table 4

| Serum Control Identification (Red) | Timothy (Blue) | Mite (Pink) | Ragweed (Green) | Cat (Purple) | Wheat (Violet) |
|---|---|---|---|---|---|
| 274 + | + | 1/0 | + | - | + |
| 891 + | + | + | + | - | + |
| 950 + | + | + | + | - | + |
| 1098 + | + | - | - | - | + |

Example 3

In this example, allergen was bound to the label particle and anti-human IgE antibody was bound to the capture line. Timothy grass allergen was coupled to label particle and monoclonal mouse anti-human IgE (Quidel) or polyclonal goat anti-human IgE (KPL Laboratories, Inc.) was bound to the capture line. Sera with known timothy grass specificity were then assayed in the assembled devices.

Individual raw or purified allergen extracts were coated overnight at room temperature at 0.5-5.0 mg/ml to colored latex (Bang's Lab's) followed by blocking with 10 mg/ml methylated BSA. The mixture was chromatographed over a Sepharose CL-4B-200 column (Sigma) to remove unbound allergen. The eluent was collected and suspended in 10mg/ml methylated BSA at 0.06-0.08% solids and was then poured onto the label zone membrane and lyophilized.

The assay device was prepared as described in example 1 except that anti-human IgE antibodies were bound to the capture zone of the device. The anti-IgE antibodies were applied to the matrix by a chart recorder in a suspension of 50 mM Tris buffer, pH 8.0. The anti-IgE antibodies were dispensed at 5 mg/ml.

The results are presented in Table 5. The device and the method provided consistent specificity for the tested antigens.

WHAT IS CLAIMED IS:

1. An assay for determining, in a biological fluid sample, the presence, absence, or amount of immunoglobulins specific for an antigen, said assay comprising:

a. applying the sample to a sample receiving zone on a matrix having a flow path to a capture zone located downstream from the sample receiving zone,

wherein the sample also flows through a means for labelling the immunoglobulins, which labelling means contains a labelling complex comprising a visible label bound to a first immunoglobulin-binding substance,

a second immunoglobulin-binding substance is immobilized in the capture zone, and

at least one of the first immunoglobulin-binding substances and the second immunoglobulin-binding substances is the antigen; and

b. observing the accumulation of visible label within the capture zone as a result of immunoglobulin present in the sample specific for the antigen specifically binding to the labelling complex in the labelling means and the resulting immunoglobulin-labelling complex flowing into and being captured within the capture zone.

2. An assay as in claim 1, wherein the biological fluid is whole blood, serum or plasma.

3. An assay as in claim 1, wherein the labelling means is located in an axial flow path between the sample receiving zone and the capture zone.

4. An assay as in claim 1, wherein the labelling means is a sample receiving pad in contact and on the surface of the sample receiving zone.

15. An assay as in claim 1, wherein the accumulation of visible label is visually compared to a reference standard.

16. An assay as in claim 15, wherein the accumulation of visible label is compared to the reference standard by a reflectance analyzer or video image analyzer.

17. An assay for determining IgE levels in a patient serum sample, said assay comprising:

applying the serum sample to a sample receiving zone on a matrix having an axial flow path defining a labelling zone located downstream from the receiving zone and a capture zone located downstream from the labelling zone, wherein a labelling complex comprising a visible label bound to a first IgE-binding substance is present in the labelling zone and a second IgE-binding substance is immobilized in the capture zone;

observing the accumulation of label within the capture zone as a result of IgE present in the patient sample specifically binding to the labelling complex in the labelling zone and the resulting IgE-labelling complex flowing into and being captured within the capture zone; and

comparing the accumulation of label to reference standards.

18. An assay as in claim 17, wherein both of the IgE-binding substances are anti-IgE, whereby the assay device measures the total IgE present in the patient serum sample.

21. A device for determining, in a biological fluid sample, levels of antigen-specific immunoglobulins reactive with an antigen, said device comprising:

a means for labelling the immunoglobulins having a labelling complex comprising a visible label bound to a first immunoglobulin-binding substance;

a matrix defining an axial flow path, said matrix having a sample receiving zone, a capture zone located downstream from the sample receiving zone, and an absorbent zone located downstream from the capture zone; and

a second immunoglobulin-binding substance immobilized in the capture zone;

whereby application of the sample to the sample receiving zone results in specific binding of immunoglobulin present in the serum to labelling complex in the labelling means and accumulation of antigen-specific immunoglobulin-labelling complex in the capture zone based on the amount of antigen-specific immunoglobulin initially present in the serum sample.

22. A device as in claim 21, wherein the labelling means is a sample receiving pad in contact and on the surface of the sample receiving zone.

23. A device as in claim 21, wherein the labelling means is a labelling zone located in the axial flow path between the sample receiving zone and the capture zone.

24. A device as in claim 21, wherein the antigen is an allergen and the immunoglobulins are IgE.

25. A device as in claim 24, wherein the first immunoglobulin binding substance is the allergen.

31. A device for determining, in a patient sample, levels of a plurality of antigen-specific immunoglobulins reactive with a plurality of different antigens, said device comprising:

at least one means for labelling the immunoglobulins, each labelling means having a labelling complex comprising a visible label bound to a first immunoglobulin-binding substance,

a matrix defining a flow path, said matrix having a sample receiving zone,

a plurality of capture zones located downstream from the sample receiving zone, and

an absorbent zone located downstream from the capture zones;

a plurality of second immunoglobulin-binding substances, each immobilized in a different capture zone;

whereby application of the patient serum sample to the sample receiving zone results in specific binding of immunoglobulin present in the serum to labelling complexes in the labelling means and accumulation of antigen-specific immunoglobulin-labelling complex in the different capture zones based on the amount of different antigen-specific immunoglobulins initially present in the serum sample.

32. A device as in claim 31, wherein the second immunoglobulin-binding substances are a Toxoplasma gondii antigen, a Rubella virus antigen, a Cytomegalovirus antigen, a Herpes Simplex virus antigen, a Chlamydia trachomatis antigen, a Treponema pallidum antigen and a Helicobacter pylori antigen and each second immunoglobulin-binding substance is immobilized in a different capture zone.

33. A device as in claim 31, wherein the first immunoglobulin-binding substance is anti-IgG or anti-IgM.

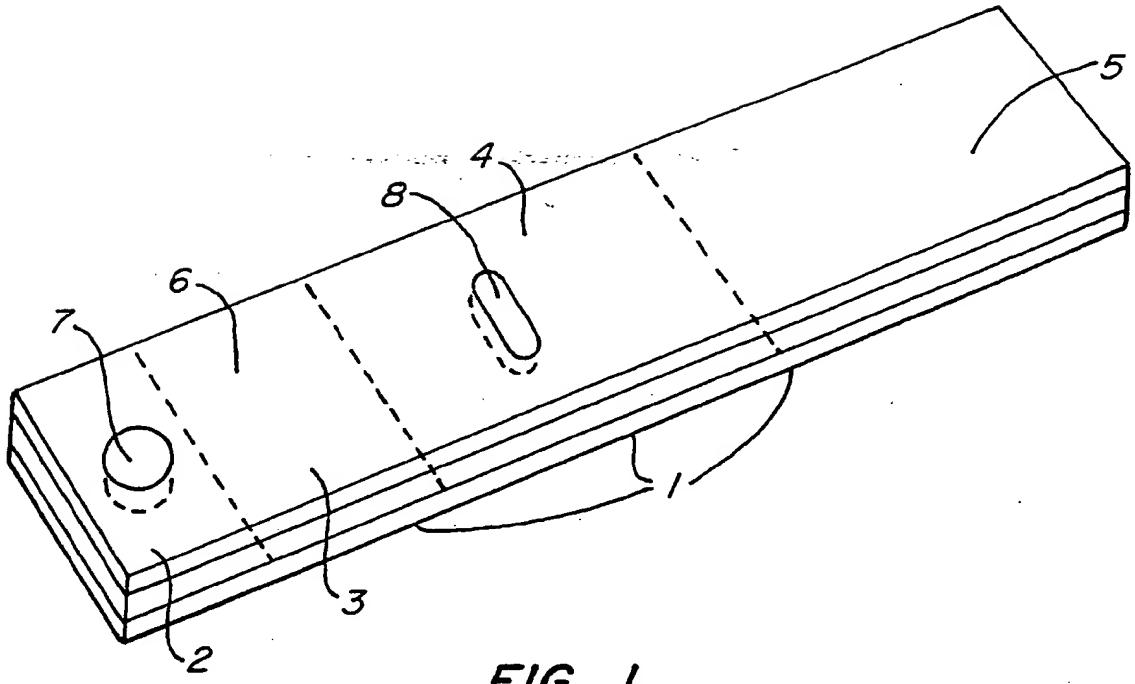



FIG. 1.

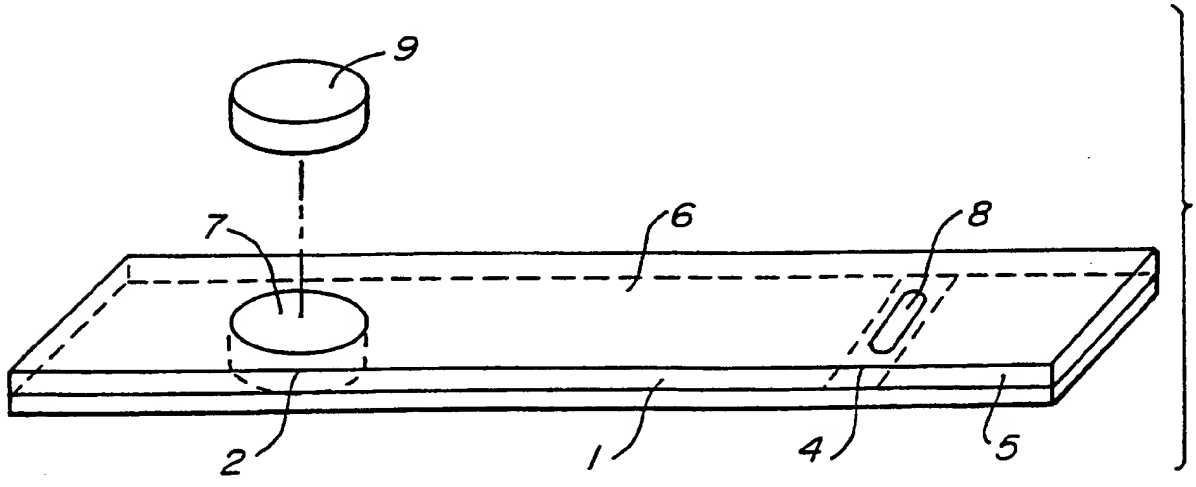



FIG. 2.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05870

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>----<br>Y | GB, A, 2,204,398 (MAY ET AL) 09 November 1988, see Figs. 1, 2 and 9; page 2, line 1; page 3, line 21 - page 4, line 2; page 4, lines 15-16 and 24-26; page 5, lines 26-34; page 6, lines 22 and 28-32; page 10, lines10-13; page 11, lines 10-17 and 22-27; page 12, line 31 - page 13, line 1; page 17, lines 6-7. | 1, 3-4, 14, 19, 21-23, 28, 31<br>----------<br>2, 5-13, 15-18, 20, 24-27, 29-30, 32-34 |
| Y | US, A, 4,943,522 (EISINGER ET AL) 24 July 1990, see Fig. 2; col. 4, lines 44-52; col. 5, lines 12-18, 28-36 and 61; col. 8, lines 8-13, 17-22 and 31-60; col. 10, lines 26-37; col. 11, lines 14-23; col. 12, lines 4-6; col. 13, line 55 - col. 14, line 7; col. 18, lines 11-18, 33, 40-41 and 53-55; Example 3. | 1-34 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>07 SEPTEMBER 1994 | Date of mailing of the international search report<br>SEP 19 1994 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>CAROL A. SPIEGEL<br>Telephone No. (703)308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05870

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

G01N 21/00, 31/00, 33/53, 33/537, 33/538, 33/541, 33/543, 33/545, 33/546, 33/553, 33/554, 33/563 ; C12Q 1/00, 1/70

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/55, 56, 57, 60; 424/11; 435/5, 7.2, 7.31, 7.32, 7.33, 7.34, 7.35; 436/169, 513, 518, 523, 525, 529, 530, 531, 533, 534, 538, 540, 541

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/55, 56, 57, 60; 424/11; 435/5, 7.2, 7.31, 7.32, 7.33, 7.34, 7.35, 805, 810, 967, 970, 971, 973, 974; 436/169, 513, 518, 523, 525, 529, 530, 531, 533, 534, 538, 540, 541, 809, 810, 811, 820